

IN THE CLAIMS

Please amend the claims as indicated in the following listing of claims, which replaces all previous listings of claims.

1. (Currently Amended) A method for DNA synthesis at high pH, comprising: a) contacting a DNA polymerase fusion with a nucleic acid template under conditions of high pH, and b) effecting template dependent synthesis of DNA, wherein said high pH ranges from 9.3 to ~~[[14]]~~ 12, and wherein said DNA polymerase fusion comprises wild type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase.

2. (Previously presented) The method of claim 1, further comprising contacting a PCR enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template.

3. (Currently Amended) A method for the cloning of a DNA synthesis product, at high pH, wherein said high pH ranges from 9.3 to ~~[[14]]~~ 12, comprising:

a) providing a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase;

b) contacting said DNA polymerase fusion with a nucleic acid template under conditions of said high pH so as to effect the template dependent synthesis of a DNA synthesis product, and

c) inserting said synthesized DNA product into a cloning vector, thereby cloning said synthesized DNA product.

4. (Previously presented) The method of claim 3, further comprising contacting a PCR enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template in step (b).

5. (Currently Amended) A method for sequencing DNA at high pH, wherein said high pH ranges from 9.3 to ~~[[14]]~~ 12, comprising the steps of:

(a) contacting a template DNA strand with a sequencing DNA primer;

(b) contacting said DNA of step (a) with a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, deoxyribonucleoside triphosphates, and a chain-terminating nucleotide analog;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize at said high pH a random population of DNA molecules complementary to said DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the

nucleotide sequence of said first DNA molecule can be determined.

6. (Original) The method of claim 5, further comprising a PCR enhancing factor and/or an additive.

7. (Currently Amended) A method of linear or exponential PCR amplification at high pH, wherein said high pH ranges from 9.3 to ~~[[14]]~~ 12, for site-directed or random mutagenesis comprising the step of: incubating a reaction mixture comprising a nucleic acid template, at least one PCR primers, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce a mutated amplified product.

8. (Original) The method of claim 7, further comprising a PCR enhancing factor and/or an additive.

9. (Currently Amended) A method of reverse transcriptase PCR at high pH, wherein said high pH ranges from 9.3 to ~~[[14]]~~ 12, comprising the step of incubating a reaction mixture comprising a nucleic acid template, at least one PCR primer, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under reaction

conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce an amplified product.

10. (Original) The method of claim 9, further comprising a PCR enhancing factor and/or an additive.

11. (Cancelled)

12. (Withdrawn) The method of claim 11, wherein said DNA polymerase fusion comprises a Glycine to Proline substitution at amino acid position 387 (G387P) and has reduced DNA polymerization activity.

13. (Previously presented) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

14. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion comprises reduced base analog detection activity and a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution.

15. (Previously presented) The method of claim 11 wherein said DNA polymerase fusion has reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

16. (Withdrawn) The method of claim 12, wherein said DNA polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

17. (Withdrawn) The method of claim 11, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

18. (Withdrawn) The method of claim 12, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

19. (Cancelled)

20. (Cancelled)

21. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

22. (Withdrawn) The method of claim 14, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

23. (Withdrawn) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

24. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion with reduced base analog detection activity further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

25. (Original) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a wild type, mutant or chemically modified DNA polymerase.

26. (Previously presented) The method of claim 1, 3, 5, 7, or 9, wherein said DNA polymerase fusion is a proofreading polymerase.

27. (Previously presented) The method of claim 26, wherein said proofreading polymerase comprises wild-type *Pyrococcus furiosus* polymerase I.

28. (Previously presented) The method of claim 1, 3, 5, 7, or 9, wherein said DNA polymerase fusion comprises an increase, as compared to said wild type *Pyrococcus furiosus* polymerase I, in at least one activity selected from the group consisting of: processivity, proofreading, fidelity, DNA binding activity, strand displacement activity, polymerase activity, nucleotide binding and recognition, efficiency, template length amplification capability, GC-rich target amplification efficiency, specificity, thermostability, intrinsic hot start capability, or salt resistance.

29. (Previously presented) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a reduction, as compared to said wild type *Pyrococcus furiosus* polymerase I, in at least one activity selected from the group consisting of: amplification slippage on templates with tri-nucleotide repeat stretches, extension time in a PCR reaction or

amplification cycles in a PCR reaction.

30. (Cancelled)

31. (Cancelled)

32. (Cancelled)

33. (Cancelled)

34. (Cancelled)

35. (Cancelled)

36. (Cancelled)

37. (Cancelled)

38. (Cancelled)

39. (Cancelled)



40. (Previously presented) The method of any one of claims 1, 3, 5, 7, and 9, wherein said DNA polymerase fusion is encoded by SEQ ID NO: 126 and has an amino acid sequence of SEQ ID NO:127.

41. (Previously presented) The method of claim 29, wherein the activity is extension time in a PCR reaction.

42. (Previously presented) The method of claim 1, wherein said high pH ranges from 9.5 to 12.

43. (Previously presented) The method of claim 3, wherein said high pH ranges from 9.5 to 12.

44. (Previously presented) The method of claim 5, wherein said high pH ranges from 9.5 to 12.

45. (Previously presented) The method of claim 7, wherein said high pH ranges from 9.5 to 12.

46. (Previously presented) The method of claim 9, wherein said high pH ranges from 9.5 to 12.

47. (Previously presented) The method of claim 42, wherein the DNA polymerase fusion is part of a blend comprising a second DNA polymerase.

48. (Previously presented) The method of claim 47, wherein the second DNA polymerase is *Pfu*.

49. (Previously presented) The method of claim 41, wherein the extension time is decreased by at least 15 seconds as compared to the extension time observed under the same conditions with the wild type Pfu polymerase.

50. (Previously presented) The method of claim 49, wherein the extension time is decreased by at least 45 seconds as compared to the extension time observed under the same conditions with the wild type Pfu polymerase.

51. (Previously presented) The method of claim 49, wherein said high pH ranges from 9.5 to 12.

52. (Previously presented) The method of claim 50, wherein said high pH ranges from 9.5 to 12.